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[71] Applicant: Panasonic Corporation

Address: Osaka, Japan

[72] Inventor:

Toshihiko Kichioka Shin Ikeda Shiro Nami

[74] Patent Agent Institution: Yong Xin Patent Brand Agent, LTD

Deputy: Cheng Wei

Claims: 3 pages

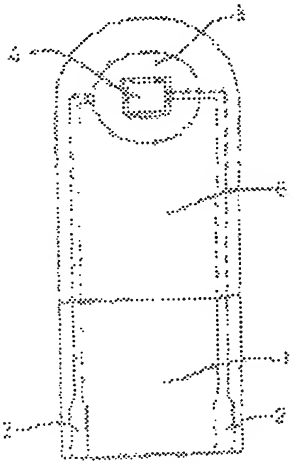
Description: 17 pages

The attached picture: 4 pages

[54] Invention Name: Biosensor

[57] Abstract:

The biosensor in this invention contains an electrically insulated main plate, an electrode system with a working electrode formed on the main plate and a counter electrode and a reaction layer formed on the main plate or above the main plate with a gap. The reaction layer contains a pyranose-oxidizing enzyme.



Claims

1. A biosensor, it includes:

An electrically insulated main plate;

An electrode system which contains a working electrode and a counter electrode;

And a reaction layer on the main plate or above the main plate with a gap;

The included reaction layer contains a pyranose-oxidizing enzyme.

2. According to the biosensor in right request 1, the included pyranose-oxidizing enzyme is pyranose oxidase (EC1.1.3.10).

3. According to the biosensor in right request 1,the included reaction layer also contains glucose oxidase (EC1.1.3.4).

4. According to the biosensor in right request 1,the included reaction layer also contains an electron receptor.

5. According to the biosensor in right request 3,the included reaction layer also contains an electron receptor.

6. According to the biosensor in right request 4, the included electron receptor is a ferricyanide ion.

7. According to the biosensor in right request 5, the included electron receptor is a ferricyanide ion.

8. According to the biosensor in right request 1, the included reaction layer contains a polysaccharide hydrolase.

9. According to the biosensor in right request 8, the included polysaccharide hydrolase is a group composed of sucrose hydrolase, maltose hydrolase and lactose hydrolase.

10. According to the biosensor in right request 1, the amount of the pyranose-oxidizing enzyme is about 1 to 200 units per cm^2 of the reaction layer.

11. According to the biosensor in right request 3, the amount of the included pyranose-oxidizing enzyme is about 0.1 to 200 units per cm^2 of the reaction layer.

12. According to the biosensor in right request 6, the amount of the pyranose-oxidizing enzyme and the ferricyanide ion are respectively about 1 to 200 units and 0.21 mg to 3.30 mg per cm^2 of the reaction layer.

13. According to the biosensor in right request 7, the amount of the pyranose-oxidizing enzyme, the glucose oxidase, and the ferricyanide ion are respectively about 0.1 to 200 units, 1 to 200 units, 0.21 mg to.30 mg per cm^2 of the reaction layer.

14. According to the biosensor in right request 1, the included biosensor is used to measure the concentration of the blood glucose.

15. A biosensor, which is used to quantitatively measure the substrate in the liquid sample, measures the substrate by reducing the electron receptor during the enzyme reaction of the substrate and by measuring the amount of reduced electron receptor with electrochemical technique, this biosensor contains:

An electrically insulated main plate;

An electrode system which contains a working electrode and a counter electrode;

And a reaction layer on the main plate or above the main plate with a gap;

The included reaction layer contains a pyranose-oxidizing enzyme and an electron receptor.

16. According to the biosensor in right request 15, the included reaction layer also contains glucose oxidase (EC1.1.3.4).

17. According to the biosensor in right request 15, the included biosensor is a glucose biosensor.

18. According to the biosensor in right request 15, the included biosensor is used to measure the concentration of blood glucose.

Description

Biosensor

This invention is a biosensor, and it can precisely, quickly, easily and quantitatively measure the substrate (specific ingredients) in the liquid sample such as blood, plasma, serum, urine, and juice. In details, this invention is a glucose sensor, and it measures the glucose concentration by interacting glucose with redoxase specifically first and then using the electrochemical technique to measure.

For quantitatively analyzing the saccharide (such as sucrose and glucose), there are various methods developed, such as spectropolarimetry, colorimetry, reductimetry and other methods using chromatographic analysis. However, none of the above methods has enough precision because of their low specificity with saccharide. Among these methods, spectropolarimetry is easy to do, and it is already known that this method is greatly influenced by the working temperature.

Recently, various biosensors are developed, and they can easily and quantitatively measure the specific ingredients (substrate) in the liquid samples such as biological samples and food, with no need to dilute and stir the liquid sample.

For example: Japanese patent publication NO.3-202746 publishes a biosensor and it contains an electrode system formed on the electrically insulated main plate, which is made with the halftone print technique and a reaction layer formed on the electrode system, which contains a hydrophilic polymer, a redoxase and an electron receptor. The following is how to use this biosensor to measure the substrate concentration in the liquid sample. First, apply the drops of the liquid sample on the biosensor reaction layer to resolve the reaction layer. This triggers the enzyme reaction between the substrate in the liquid sample and the redoxase, and the electron receptor in the reaction layer is

reduced. After the enzyme reaction, oxidize the reduced electron receptor, so the substrate concentration in the liquid sample is measured according to the obtained oxidization current during the oxidization process.

America patent No. 5,192,415 publishes a biosensor, which contains a control layer for proton concentration, and this layer does not need to preset the pH in the liquid sample because it can optimize the pH in the liquid sample according to the kind of redoxase in the reaction layer.

America patent No. 5,264,103 publishes a biosensor, which contains a major electrode system on the electrically insulated main plate, which has a working electrode and a counter electrode, a reaction layer with a kind of redoxase, and a sub-electrode layer, which has a gap from main electrode system and contains a working electrode and a counter electrode.

These biosensors have very broad application, for example, by appropriate choosing the redoxase in the reaction layer, the biosensor can turn into the glucose sensor, the ethanol sensor, the cholesterol sensor or the amino acid sensor.

Among these biosensors, it is well known the glucose sensor has glucose oxidase as redoxase. However, this glucose sensor has the following problems. Among the various isomers of glucose, glucose oxidase only react with β-glucose and in equilibrium, the percentage of β-glucose is 63%. Therefore, the responding current value obtained by this glucose sensor (in other words, measuring sensitivity) is too low, which causes big error when quantitatively measuring very little amount of glucose.

In addition, when measuring the polysaccharide with this biosensor, the major glucose produced by hydrolase is α-glucose, so before quantitative measurement, an extra step is needed: use a mutarotase to turn the α-glucose into its isomer β-glucose.

Japanese patent application No. 6-291401 is a biosensor with mutarotase and glucose oxidase. However, in this biosensor, when the total amount of these enzymes is low, the measuring sensitivity cannot be improved enough, when the total amount is high, the production cost will increase. In addition, when the substrate concentration in the liquid sample is high, the biosensor with the mutarotase and the glucose oxidase has lower sensitivity than the biosensor without the mutarotase.

The biosensor in this invention contains: an electrically insulated main plate; an electrode system on the main plate and with a working electrode and a counter electrode; and a reaction layer on the main plate or above the main plate with a gap. The reaction layer contains one pyranose-oxidizing enzyme.

In one implementation scheme, the pyranose-oxidizing enzyme is pyranose oxidase (EC1.1.3.10).

In other implementation scheme, the reaction layer also has glucose oxidase (E.C.1.1.3.4).

In another implementation scheme, the reaction layer also has an electron receptor.

In another implementation scheme, the electron receptor is a ferricyanide ion.

In another implementation scheme, the electron receptor also has polysaccharide hydrolase.

In another implementation scheme, the polysaccharide hydrolase is a group composed of free sucrose hydrolase, maltose hydrolase and lactose hydrolase.

In one implementation scheme, the amount of the pyranose-oxidizing enzyme is about 1 to 200 units per cm^2 of the reaction layer.

In another implementation scheme, the amount of the pyranose-oxidizing enzyme is about 0.1 to 200 units per cm^2 of the reaction layer.

In another implementation scheme, the amount of the pyranose-oxidizing enzyme and the ferricyanide ion is about 1 to 200 units and about 0.21 mg to 3.30 mg respectively per cm^2 of the reaction layer.

In another implementation scheme, the amount of the pyranose-oxidizing enzyme, glucose oxidase and the ferricyanide ion is about 0.1 to 200 units per cm^2 of the reaction layer, about 1 to 200 units and 0.21 to 3.30 mg per cm^2 of the reaction layer respectively.

In one implementation scheme, the biosensor is used to measure the level of blood sugar (glucose).

Or, the measuring principle for this invention to quantitatively measure the substrate in the liquid sample is: reduce electron receptor with the electrons produced during the substrate's enzyme reaction, and use electrochemical technique to measure the amount of the electron receptor still in reduction state. The biosensor contains: an electrically insulated main plate; an electrode system on the main plate and with a working electrode and a counter electrode; and a reaction layer on the main plate or above the main plate with a gap. The reaction layer contains a pyranose-oxidizing enzyme and an electron receptor.

In one implementation scheme, this reaction layer also contains glucose oxidase.

In another implementation scheme, this biosensor is a glucose sensor.

In another implementation scheme, this biosensor is used to measure the level of blood sugar (glucose).

Therefore, the described invention makes the following virtues possible:

(1) Provide a biosensor which can measure the substrate concentration in the liquid sample precisely and quickly through simultaneously measure α-glucose and β-glucose; (2) provide a biosensor which can easily measure the substrate concentration in the liquid sample with polysaccharide; and (3) provide a low-cost biosensor.

To the persons familiar with this technical field, when they read and understand the detailed introduction in the following attached pictures for reference, those virtues and other virtues of this invention become obvious.

Followed is the brief introduction for the attached pictures:

Picture 1 is the principle ichnography of the biosensor as an example of this invention; the reaction layer is omitted;

Picture 2 is the principle sectional view of the biosensor as an example of this invention, the reaction layer directly located on the main plate.

Picture 3 is the principle sectional view of the biosensor as an example of this invention; the reaction layer is above the main plate with a gap.

Picture 4 is the plot showing the relationship between the glucose concentrations and the responding current values, which are from an example of this invention and the examples of other biosensors for comparison. Curve (a) shows the change of the responding current values with the reaction layers having PyOx and GOD simultaneously, curve (b) shows the change of the responding current values with the reaction layers only having GOD, curve (c) shows the change of the responding current values with the reaction layers only having PyOx.

Picture 5 is the plot showing the relationship between the blood sugar (glucose) concentration and the responding current values, which are from an example of this invention and the examples of other biosensors for comparison. Curve (a) shows the change of the responding current values with

the reaction layers having PyOx and GOD simultaneously, curve (b) shows the change of the responding current values with the reaction layers only having GOD, curve (c) shows the change of the responding current values with the reaction layers only having PyOx.

The biosensor contains: an electrically insulated main plate; an electrode system on the main plate and with a working electrode and a counter electrode; and a reaction layer on the main plate or above the main plate with a gap.

The electrically insulated plate is made of a synthetic resin, such as polyethylene terephthalate, polyethylene, polyester, polypropylene, polystyrene, and polyvinyl chloride.

The electrode system with the working electrode and the counter electrode can form on the main plate using the methods already known. For example, after the formation of down-leads on the main plate, the working electrode and the counter electrode are formed and connected with their down-leads and insulated with each other. The material of the down-leads and electrodes can be any conducting material, such as carbon, silver, platinum, gold and palladium.

The reaction layer of this biosensor contains a pyranose-oxidizing enzyme, which can simultaneously oxidize α-glucose and β-glucose. An example of this pyranose-oxidizing enzyme is PyOx (EC1.1.3.10).

The optimal amount of the PyOx in the reaction layer of this biosensor is about 1 to 200 units per cm^2 of the reaction layer, the more optimal amount is about 2 to 50 per cm^2 of the reaction layer. The "unit" here is the amount of redoxase required to oxidize 1 μmol glucose or polysaccharide in one minute. When the amount of PyOx is less than 1 unit per cm^2 of the reaction layer, extra minutes or extra measuring time is needed. Moreover, because of the evaporation of the liquid sample within

the extra time, the responding current value may be affected. More than 200 units per cm^2 of the reaction layer not only increase the product cost, but also causes unstable responding current value because of the damage during the formation of the reaction layer.

To further improve the measuring sensitivity for the glucose in the liquid sample and enable glucose sensor to respond a broader glucose concentration range, the reaction layer has GOD (EC1.1.3.4) in addition to PyOx. The optimal amount of the GOD in the reaction layer of this biosensor is about 1-200 units per cm^2 of the reaction layer. When GOD and PyOx are used together, the optimal amount of the PyOx is about 0.1 to 200 units per cm^2 of the reaction layer, the more optimal amount is about 0.2 to 40 per cm^2 of the reaction layer.

The reaction layer can also contain various hydrophilic polymers. The examples of these polymers are: carboxyl methylbenzene cellulose (use CMC in the following), hydroxyl ethide cellulose (HEC), hydroxyl propyl cellulose (HPC), methyl cellulose, ethide hydroxyl ethide cellulose, carboxyl methyl ethide cellulose, plasmosan, polyvinyl alcohol, polyamino acid such as polylysine, ploystyrene sulfonic acid, latex and its derivative, acrylic acid and its salts methacrylic acid and its salts, starch and its derivative, maleic anhydride and its salts, particularly, the optimal option is CMC.

When applying the liquid sample with the glucose on this biosensor, α-glucose and β-glucose are oxidized by PyOx respectively. At the same time, the oxygen in the liquid sample is reduced to the peroxide. If the voltage is applied at this time, the peroxide is oxidized. The responding current value during the oxidization process is proportional to the peroxide concentration, in other words, proportional to the substrate concentration in the liquid sample. So, the substrate concentration in the liquid sample can be obtained through measuring responding current value.

In this biosensor, the reaction layer can have an electron receptor, in this case, the peroxide will not be produced during the substrate oxidization process, instead, the reduction state of electron receptor is formed during the enzyme reaction. The examples for the electron receptors include: diferricyanide ion, p-benzoquinone and its derivative, phenazine methosulfate, methylene blue, dicyclopentadienyl iron and its derivative. One or several kinds of electron receptor can be used. Particularly, the optimal option is the ferricyanide ion.

The optimal amount of the ferricyanide ion is about 0.21 to 3.30 mg per cm^2 of the reaction layer, the more optimal amount is about 0.30 to 2.59 mg per cm^2 reaction layer. When the ferricyanide ion amount is less than 0.21 mg per cm^2 of the reaction layer, the measurable glucose concentration range may be very small. When the amount of the ferricyanide ion is more than 3.30 mg per cm^2 of the reaction layer, the responding current value may be unstable because of the damage during formation the reaction layer, and the reliability of this biosensor may decrease during storage.

The reaction layer of this biosensor can also contain a polysaccharide hydrolase, which can be used to hydrolyze polysaccharide to produce α-glucose. Polysaccharide hydrolase is an enzyme, which can hydrolyze polysaccharide such as sucrose and maltose to produce glucose. The examples for this kind of polysaccharide hydrolase are: sucrose hydrolase such as invertase (use INV in the following), maltose hydrolase such as maltase, and lactose hydrolase such as β- galactosidase. The optimal amount of the polysaccharide hydrolase is about 1 to 400 units per cm^2 reaction layer, the more optimal amount is about 2 t0 200 per cm^2 of the reaction layer.

The following is how to make this biosensor by referring to Picture 1 and Picture 2.

First, print conducting material such as liquid sliver on the electrically insulated main plate using the halftone print method, to form down-lead 2 and 3. Then print another conducting material with the resin adhesive on the main plate 1, to form a working electrode 4, and connect it with down-lead 2.

Then print the insulation liquid on the main plate 1, so the insulation layer 6 is formed. The insulation layer 6 covers the surrounding area of the working electrode 4, only a fixed area of the working electrode is exposed. As Picture 1 shows, insulation layer 6 also covers a part of down-lead 2 and down-lead 3. Surrounding the working electrode 4 is the counter electrode 5 with ring shape made of the conducting material with the resin adhesive. The counter electrode 5 connects with down-lead 3. Therefore, the electrode system 8 including a working electrode 4 and the counter electrode 5 is formed on main plate 1.

Or, this biosensor can also contain a trielectrode system formed on the main plate 1, which includes a reference electrode (not shown) besides the working electrode 4 and the counter electrode 5. The aim for the trielectrode system is to further stabilize the measuring precision.

The reaction layer forms on main plate 1 according to the following method:

Apply the drops of the water solution of the hydrophilic polymer on the electrode system 8 and let them dry, so a hydrophilic polymer layer is formed. Then, apply the drops of water solution with PyOx and when needed with electron receptor and/or polysaccharide hydrolase, and let them dry. To use this biosensor repeatedly, the enzymes can associate with glutaric dialdehyde and be fixed on the hydrophilic polymer, or the pyranose-oxidizing enzyme and when needed polysaccharide hydrolase can be fixed on the hydrophilic polymer together with the polymer material, the included polymer material can, for example, be: pyroxylin, cellulose acetate, and polyacrylonitrile. Moreover, if needed, the electron receptor can be fixed on the hydrophilic polymer using polymer material and through chemical method. Therefore, as shown in Picture 2, a reaction layer 7 covering all the electrode system 8 is formed.

Alternatively, the reaction layer can be located above main plate 1, with a gap from main plate 1. In this situation, as shown in Picture 3, this biosensor contains a main plate 1 and a covering plate 30 above the main plate 1, between them is an insulator 20.

The covering plate contains a hole 31 and a reaction layer 37 formed on one side of its surface. The covering plate 30 is above the main plate 1, and is facing the reaction layer 37 and electrode system 8. The reaction layer 37, which is above the main platel and with a gap from the main plate 1, can be formed as the described in Japanese Patent Open No.1-114747. In this kind of biosensor, when the applied sample, through one sampling hole 38, reaches the space between the reaction layer 37 and the electrode system 8, the amount of the peroxide produced by the reaction layer 37 or the amount of the electron receptor in reduction state can be measured out by the electrode system 8 through the same way as the biosensor shown in Picture 2.

This biosensor can be used to quantitatively measure various substrates in biological samples, these samples, for example, are: the whole blood, plasma, serum, urine, the materials and the products such as the fruit juice in food industry. Particularly, when measuring the level of the blood sugar (glucose) of the particular patient, this biosensor can be used as the easy-to-use and one-off sensor for the blood sugar (glucose).

Application examples:

Now, we will introduce some specific examples of this biosensor. Please note this biosensor is not limited to these examples. In the attached picture of each example, the same component is represented with the same label, and to be concise, a part of introduction is omitted.

Application example 1:

As an example of this biosensor, a glucose sensor is made using the following method.

As shown in Picture 1, use the halftone print method to print the liquid silver on the electrically insulated main plate, which is made of polyethylene terephthalate, and form the down-lead 2 and 3.

Then, print the conducting liquid carbon with the resin adhesive on the main plate 1 to form a working electrode 4, and connect it to the down-lead 2.

Then, print the insulation liquid on the main plate 1 to form an insulation layer 6. The insulation layer 6 covers the surrounding part of the working electrode 4, and a fixed area of the working electrode 4 is exposed.

Then, print the conducting carbon liquid with the resin adhesive on the main plate 1 to form a circle electrode 5, and connect it to the down-lead 3.

Apply the drops of the 0.5% (weight) water solution of CMC on the electrode system 8, in other words, on the working electrode 4 and the counter electrode 5 and let them dry, so that a CMC layer is formed. Apply the drops of a mixture with PyOx and ferricyanide potassium on the CMC layer and let them dry, so that a reaction layer 7 is formed. The amount of the PyOx and frricyanide potassium in the reaction layer is respectively 10 units and 1.3 mg per cm^2 of the reaction layer.

Apply the drops of the 90 mg/dl glucose water solution, which is used as the liquid sample, on the reaction layer 7 of the glucose biosensor made in this way. After one minute, apply the voltage of +0.5V from the working electrode 4 to the counter electrode 5, and measure the current value 5 seconds after the application of the voltage. In this way, measure the responding current values in the glucose solution for 12 times, and every time, a new glucose sensor is used. The fluctuation of the obtained responding current value is small.

Moreover, in the same way as the above, measure the responding current values in the 180 mg/dl and 360 mg/dl glucose water solution for 12 times respectively. It is found that the responding current values obtained in this way increase with the increase of the concentration of the glucose and the ratio of the increase is big.

As a comparative example, a glucose sensor with glucose oxidase (EC1.1.3.4) instead of PyOx is made and is used to measure the responding current values for 12 times for the above glucose solutions with various concentrations. On the same concentration of the glucose, the obtained

responding current values are unstable. In addition, although it is found that the responding current values increase with the increase of the glucose concentration, the ratio of the increase is small.

Application example 2

As an example of this biosensor, a sucrose sensor is made using the following method.

Using the same method to show in Application example 1, from the down-lead 2,3, the electrode system 8 (a working electrode 4 and a counter electrode 5) and the insulation layer 6 on the electrically insulated main plate, which is made of polyethylene terephthalate. Then apply the drops of 0.5% (weight) water solution of CMC on the electrode system 8 and let them dry, so that a CMC layer is formed.

Apply the drops of a mixture containing PyOx, INV and ferricyanide potassium and let them dry, so that the reaction layer 7 is formed. The amount of PyOx, INV, and ferricyanide potassium in the reaction layer 7 is 10 units, 40 units and 1.3 mg per cm^2 of the reaction layer.

Apply the drops of the 171 mg/dl water solution of the sucrose on the reaction layer 7 of the sucrose sensor made in this way, which are used as the liquid sample. The reaction layer 7 is resolved by this liquid sample. After 3 minutes, apply +0.5V voltage from the working electrode 4 to the counter electrode 5, and measure the current value 5 seconds after the application of the voltage.

Moreover, measure the responding current values for the 342 mg/ dl and 684 mg/dl water solution of the sucrose with the same method as the above, and use a new sucrose sensor for every measurement.

It is found that the responding values increase with the increase of the sucrose concentration, and the ratio for the increase is big.

As comparative examples, a maltose sensor and a lactose sensor are made, the making methods are the same as the described before except that INV is replaced with the maltose hydrolase and the

lactose hydrolase respectively.

Application example 3

As an example of this biosensor, a sucrose sensor is made using the following method.

Using the same method to show in Application example 1, from the down-lead 2,3, the electrode system 8 (a working electrode 4 and a counter electrode 5) and the insulation layer 6 on the electrically insulated main plate, which is made of polyethylene terephthalate. Then apply the drops of 0.5% (weight) water solution of CMC on the electrode system 8 and let them dry, so that a CMC layer is formed.

Apply the drops of a mixture containing PyOx, GOD and ferricyanide potassium and let them dry, so that the reaction layer 7 is formed. The amount of PyOx, GOD, and ferricyanide potassium in the reaction layer 7 is 1 unit, 10 units and 1.3 mg per cm^2 of the reaction layer.

Apply the drops of the 90 mg/dl water solution of the sucrose on the reaction layer 7 of the sucrose sensor made in this way, which are used as the liquid sample. After 1 minute, apply +0.5V voltage from the working electrode 4 to the counter electrode 5, and measure the current value 5 seconds after the application of the voltage.

Moreover, measure the responding current values for the 180 mg/ dl and 360 mg/dl water solution of the sucrose with the same method as the above, and use a new sucrose sensor for every measurement. The responding feature between the glucose concentrations and the responding current values is shown on curve (a) in Picture 4.

As comparative examples, a glucose sensor without PyOx and a glucose sensor without GOD are made, and the responding current values are measured as the described before. The results are shown on curve (b) and curve (c) in Picture 4.

As shown in Picture 4, the glucose sensor only having GOD (corresponding to the curve (b) in Picture 4) gets the lowest responding current value, this is because at this time, the responding current values only depend on the concentration of the β-glucose in the liquid sample. In comparison, The glucose sensor only having PyOx as the enzyme (corresponding to the curve (c) in Picture 4), gets the higher responding current values than the curve (b), particularly when the concentration of glucose is low because at this time the responding current values depend on the combined concentration of the α-glucose and β-glucose. But, when the concentration the glucose is high, the glucose sensor only having PyOx gets the lower responding current values than the curve (b). In result, the glucose sensor simultaneously having PyOx and GOD (corresponding to the curve (a) in Picture 4), always gets the high responding current value within the broadest concentration range.

Application example 4

As an example of this biosensor, a sucrose sensor is made using the following method.

Using the same method to show in Application example 1, from the down-lead 2, 3, the electrode system 8 (a working electrode 4 and a counter electrode 5) and the insulation layer 6 on the electrically insulated main plate, which is made of polyethylene terephthalate. Then apply the drops of 0.5% (weight) water solution of CMC on the electrode system 8 and let them dry, so that a CMC layer is formed.

Apply the drops of a mixture containing PyOx, GOD, INV and ferricyanide potassium and let them dry, so that the reaction layer 7 is formed. The amount of PyOx, GOD, INV, and ferricyanide potassium in the reaction layer 7 is 1 units, 10 units, 40 units and 1.3 mg per cm^2 of the reaction layer.

When the drops of the 171 mg/dl water solution of the sucrose are applied on the reaction layer 7 of the sucrose sensor made in this way, the reaction layer 7 is resolved by this liquid sample. After 3 minutes, apply +0.5V voltage from the working electrode 4 to the counter electrode 5, and measure the current value 5 seconds after the application of the voltage.

Moreover, measure the responding current values for the 342 mg/ dl and 684 mg/dl water solution of the sucrose with the same method as the above, and use a new sucrose sensor for every measurement.

It is found that the responding values increase with the increase of the sucrose concentration, and the current values, within a broad concentration range of the sucrose, are always high.

As comparative examples, a sucrose sensor is made as the described above, except that there is no GOD in the reaction layer, then measure the responding current value with the similar method. The obtained responding current values are always lower than the current values obtained by the sucrose sensor with GOD.

The maltose sensor and the lactose sensor are made by replacing INV with the maltose hydrolase and the lactose hydrolase, it is found that they show similar effects as the above.

Application example 5

Using the same method to show in Application example 1-4, some biosensors are made except that there is no ferricyanide potassium in the reaction layer 7. Apply the drops of the liquid sample with various substrate concentrations as in Application example 1-4 on the electrode systems of these biosensors. After a preset period, apply +1.0V voltage from the working electrode 4 to the counter electrode 5, and measure the current value 5 seconds after the application of the voltage.

It is found that the responding values increase with the increase of the sucrose concentration, and the current values.

Application example 6

Using the same method to show in Application example 3, a glucose sensor is made.

Apply the drops of the whole blood with 95 mg/dl glucose on the reaction layer 7 of the glucose sensor made in this way, which are used as the liquid sample. After 1 minute, apply +0.5V voltage from the working electrode 4 to the counter electrode 5, and measure the current value 5 seconds after the application of the voltage.

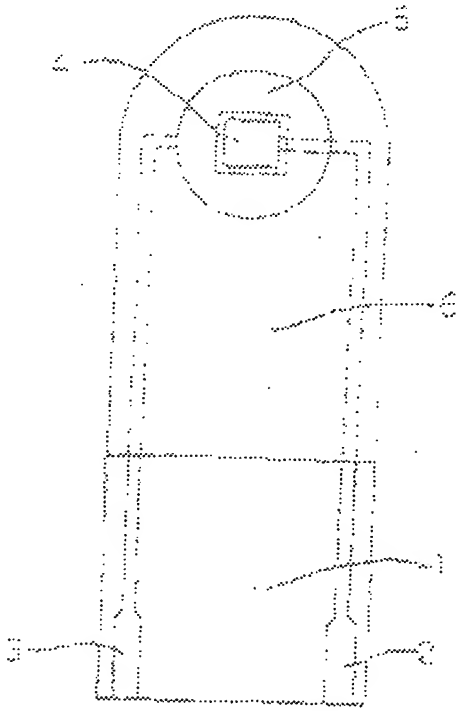
Moreover, measure the responding current values for the whole blood with 170 mg/ dl and 320 mg/dl glucose with the same method as the above, and use a new sucrose sensor for every measurement. The responding feature between the glucose concentrations and the responding current values is shown on curve (a) in Picture 5.

As comparative examples, a glucose sensor without PyOx and a glucose sensor without GOD are made, and the responding current values are measured as the described before. The results are shown on curve (b) and curve (c) in Picture 5.

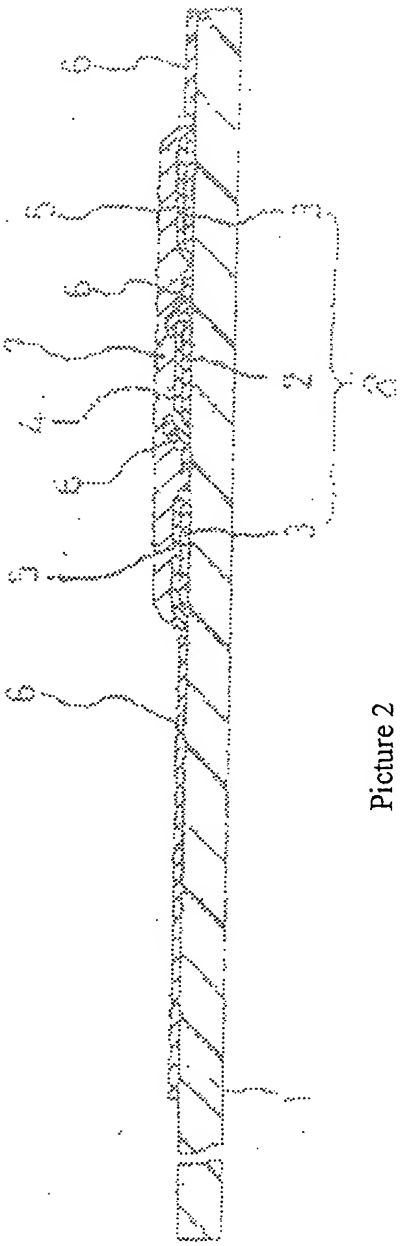
As Picture 5 show, the glucose sensor only having GOD presents the lowest responding current value (corresponding to curve (b)), this is because the responding current values only depend on the concentration of the β-glucose in the whole blood. In comparison, the glucose sensor only having PyOx presents the higher responding current value than the responding current value in curve (b) (corresponding to curve (c)), this is because the responding current values depend on the sum of the concentrations of the α-glucose and β-glucose in the whole blood. In result, the glucose sensor simultaneously with PyOx and GOD (corresponding to curve (a) in Picture 5) always presents the high responding current value within the broadest concentration range of the blood glucose.

To people familiar with the techniques in this field, they can obviously understand and easily fulfill other modifications within the category and idea of this invention. Therefore, we don't wish to restrict the range of the attached Claim to the applications presented here, and we wish to these Claim to be comprehended in the broad meaning.

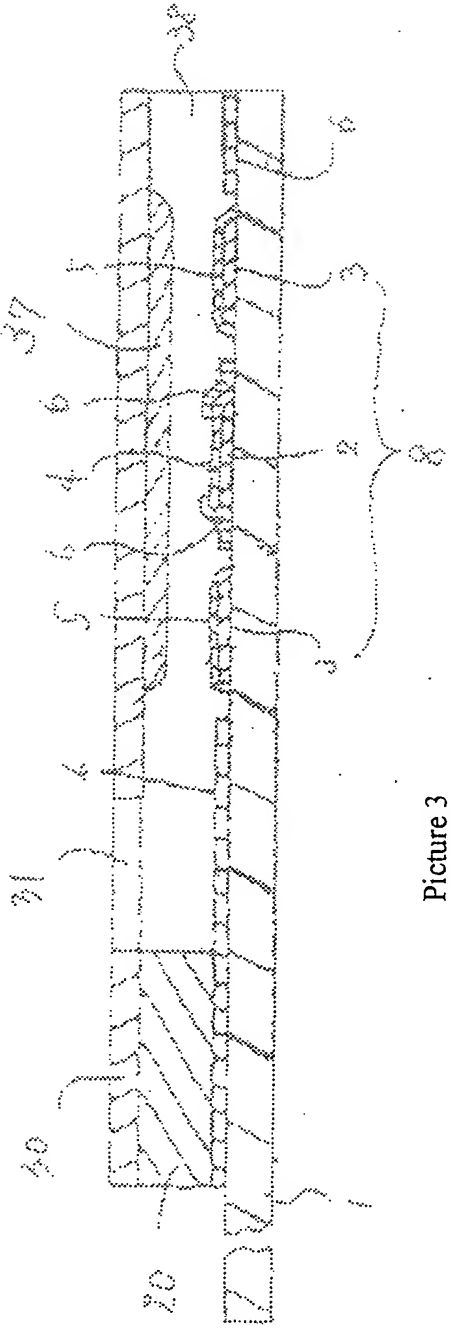
The Attached Picture of the Description



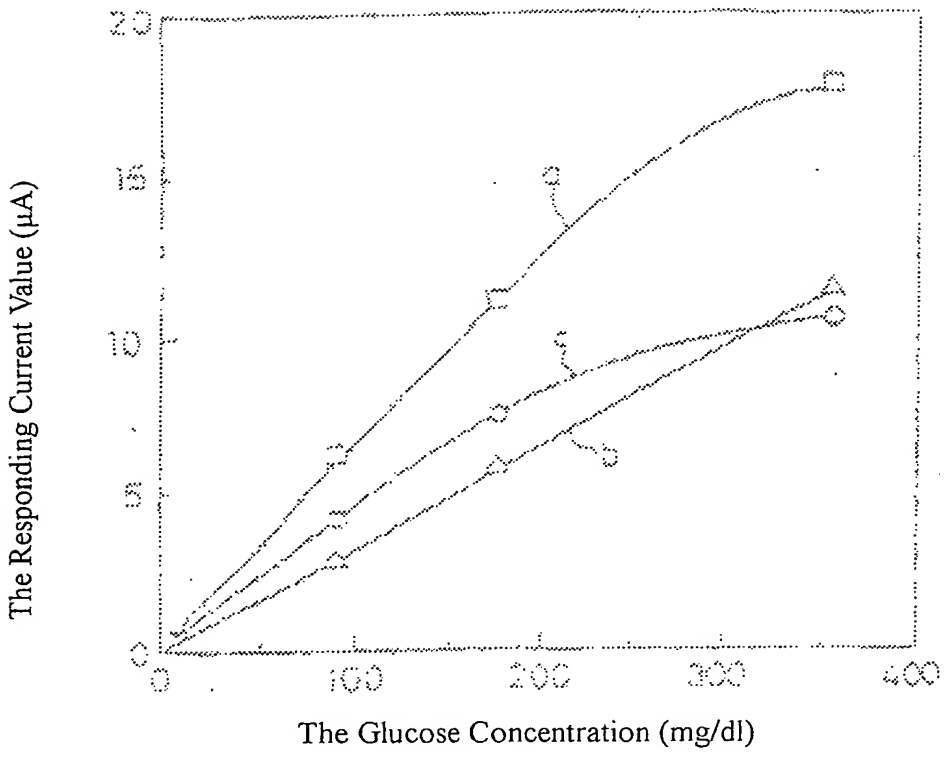
Picture 1



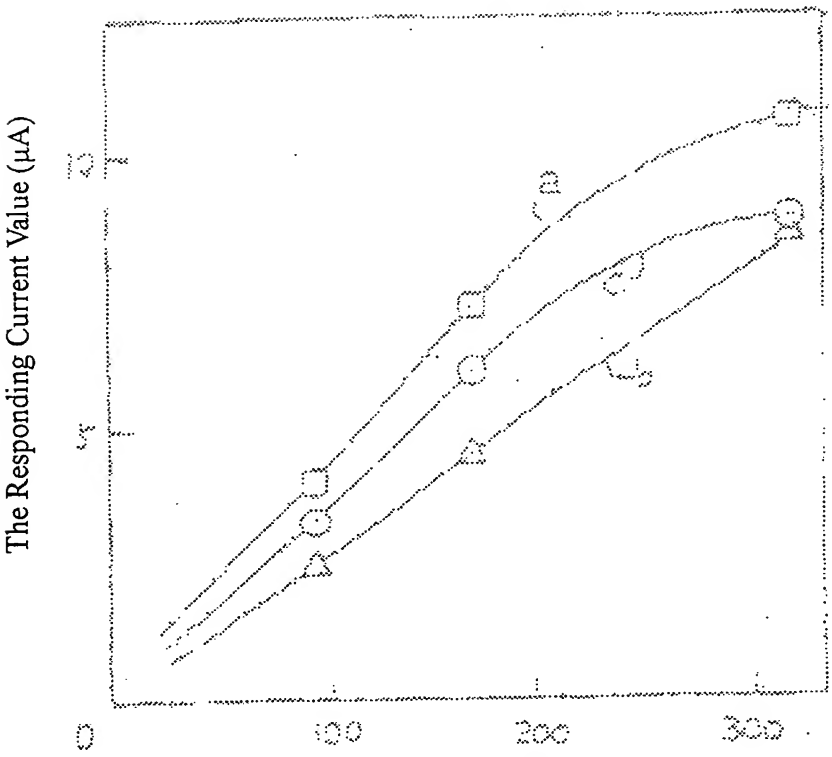
Picture 2



Picture 3


The Responding Current Value (μA)
20
15
10
5
0
0
100
200
300
400
The Glucose Concentration (mg/dl)

Picture 4


The Responding Current Value (μA)
10
5
0
100
200
300
a
b
c
The Glucose Concentration (mg/dl)

Picture 5